Journal of Agriculture and Environment for International Development - JAEID 2013, 107 (2): 201 - 212
DOI 10.12895/jaeid.20132.138

Evaluation of the incidence and severity of Olive Leaf Spot caused by *Spilocaea oleagina* in different olive cultivars in Palestine

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Presented on 2013, 15 March, accepted on 2013, 5 September. Section: Research Paper

Abstract: Twelve olive (*Olea europaea* L.) cultivars including Nabali Baladi, Nabali Mohassan, Santa Augustino, Nasohi Jaba`a 1, Nasohi Jaba`a 2, Yunani, Talmasani, Chemlali, Arbequino, Frantoio, Coca and Barouni, were sampled for their susceptibility to *Spilocaea oleagina* the causal agent for Olive leaf spot (OLS) disease on olive trees in Palestine. Investigations were carried out to measure the incidence and severity of the disease at Qabatyia station in Jenin district area. Susceptible olive cultivars grown commercially in Palestine include Arbequino, Frantoio and 'Barouni'. The "Nabali" is the most dominant and highly susceptible olive cultivar grown in Palestine. Disease incidence varied greatly among the cultivars $F=59.4$, $df=11, 251$, $p>0.0001$ and was correlated with the severity ($y = 0.42x + 9.3$, $P \leq 0.0001$, $R^2 = 0.84$, y = disease severity and x = disease incidence). Disease severity also varied among different cultivars $F=13.9$, $df=11, 251$, $p>0.0001$. Nabali Mohassan was the most affected susceptible while Barouni was most resistant. Progress of disease severity over time fit the logistic function for all cultivars except for highly susceptible cultivars $F=1.56$, $df=6, 83$, $p>0.169$. The assessment method may be useful to screen olive cultivars for OLS resistance in Palestine.

Keywords: olive cultivars, Spilocaea oleagina*, susceptibility, disease incidence, disease severity.*

Introduction

Olive (*Olea europaea* L.) is one of the oldest agricultural tree crops which are cultivated over large areas in Palestine. The number of olive trees is exceeding more than 10 million (67.3% of all horticultural trees in the Palestinian Territory on

October 1, 2011) and occupying more than 50% of the agricultural area. Olive production contributes to about 12-13% of the national income (Palestinian Central Bureau of Statistics (PCBS) 2012).

The olive tree is affected by many pests and diseases (Sergeeva *et al.*, 2008; MacDonald *et al.*, 2000; Sanei and Razavi, 2011). Olive leaf spot (OLS) is a foliar disease widespread in all olive growing regions of the world, and has been known in the Mediterranean areas (Obanor *et al.*, 2005; Shabi *et al.*, 1994; Graniti, 1993). Olive leaf spot (OLS), also known as peacock spots, is caused by the fungus *Spilocaea oleagina* (Cast.) Hughes (syn. *Cycloconium oleaginum* Cast.) (Gonzalez-Lamothe *et al.*, 2002). Severely infected trees show defoliation, poor twig and growth. As a result of infection, yield losses may reach up to 20% (Azeri, 1993; Graniti, 1993). Symptoms of the disease occur usually on the upper surface of the leaves, expanding and coalescing to cover a large proportion of leaf area. The lesions form dark brown round spots (2-15 mm in diameter) which become necrotic and surrounded by concentric yellowish or pale brown haloes (Sanchez *et al.*, 1998). Leaf spot is usually more abundant on the lower parts of olive trees (Razavi and Jahany, 2009; Azeri, 1993; Graniti, 1993). Infection with *S. oleagina* is normally associated with high humidity, where high temperatures restrict spore germination (Obanor *et al.*, 2008a; Al-Khatib *et al.*, 2010). The growth of *S. oleagina* is found to be most prevalent in the period from late autumn to spring (Viruega and Trapero, 1999; Obanor *et al.*, 2008a) and of minor significance in the period from the beginning of July until the middle of November. OLS infections can occur at any time of the year, but usually during late autumn through to early summer if environmental conditions are favourable. Infections then become evident in spring. In hot dry weather conditions, conidia remain viable but inactive on infected leaves and start to germinate early in winter. Conidial production is optimal at 15ºC and or temperatures ranging from 2 to 25 ºC and high humidity (100%) (Obanor *et al.*, 2008a; Graniti, 1993; Guechi and Girre, 1994). Conidia of *S. oleagina* are dispersed by rain splash or wind-borne water droplets (De Marzo *et al.*, 1993).

The disease is chemically controlled by application of coppers fungicides directly after harvesting (Obanor *et al.*, 2008b; Sistani *et al.*, 2009). The most commonly used fungicides include Bordeaux mixture, copper hydroxide, copper oxide and copper oxychlorides, although some long-persisting preventative fungicides such as chlorothalonil and dodine have also been used to control the disease (Sistani *et al.*, 2009). Chemical treatment appears to be rarely effective (Graniti, 1993; Obanor *et al.*, 2008b). Moreover, using chemical fungicides leads to the appearance of resistant pathogen races to Cu (Vanneste *et al.*, 2003; Carisse *et al.*, 2000) as well as disturbance of the plant metabolism following Cu accumulation in the soil (Obanor *et al.*, 2008b).

In recent years, alternative techniques have been applied for the control of the disease. Genetic resistance represents an effective, economically feasible and

ecologically sustainable mean to control the peacock disease (Rhouma *et al.*, 2012; Sanei and Razavi, 2011; Zine El Aabidine *et al.*, 2010). However, the level of susceptibility of olives to OLS is widely variable (Graniti, 1993; Sutter, 1994). Several varieties (e.g Leccino and Valatolina) are resistant to *S. oleagina* (MacDonald *et al.*, 2000; Sanei and Razavi, 2011). On the other hand, some varieties such as Arbequine, Frantoïo, Barouni and Nabali are sensitive (MacDonald *et al.*, 2000; Sutter, 1994).

In Palestine, the disease is wide common in many areas including Hebron, Bethlehem, Ramallah, Nablus, Qalqilia, Tulkarm, Salfit, Jenin and Tubas (Salman *et al.*, 2011). Susceptible olive cultivars grown commercially in Palestine include "Arbequino", "Frantoio" and "Barouni" (Bartolini and Cerreti, 2008). The "Nabali" is the most dominant grown olive cultivar in Palestine; this cultivar is highly susceptible to the leaf-spot disease. Limited data on olive leaf spot disease in Palestine especially the susceptibility of cultivars to the disease are available. The aim of this study in general, was to evaluate the resistance of Olive cultivars to OLS disease, grown in Jenin discrete (Qabatyia Station). So that resistant cultivars can be identified and thus used for replanting, or as sources for resistance in future breeding programmes.

Materials and Methods

Sample collection

A field survey was conducted during the summer season of 2012. About 80 olive trees (about 30 years old) of different cultivars (nearly the same age) at Qabatyia/Jenin district area (32.28N, 35.18E), were collected monthly from April to October. The surveyed grove included 12 olive cultivars (Nabali Baladi, Nabali Mohassan, Santa Augustino, Nasohi Jaba`a 1, Nasohi Jaba`a 2, Yunani, Talmasani, Chemlali, Arbequino, Frantoio, Coca and Barouni).

Determination of disease incidence and severity

Five randomly trees of each cultivar were surveyed and three samples (300 leaves) were collected. Leaf samples were collected according to Salman et al (2011). On each tree, 100 leaves on the surrounding four sides from the medium portion (1.5 meter above the grund) of canopy of the tree were randomly selected. Leaves with visible and invisible symptoms were collected for evaluation of disease incidence and severity in the laboratory by immersing the leaves in 5% NaOH for 2 minutes at 50-60°C (Shabi *et al.*, 1994). Disease incidence was assessed by determining the percentage of infected leaves. From visible lesions on the leaves a sub-sample (25 leaves) were taken randomly to evaluate the severity of peacock spot by visually estimating the area (%) covered with lesions and counting the number of lesions on each leaf. Severity was

recorded as 5, 12.5, 25, 37.5, 50, 75, 90 and 100% area covered with OLS. The number of lesions were graded 1 (1 lesion), 2 (2 lesions), 3 (3-5 lesions), 4 (6-10 lesions) or 5 (11+ lesions) (MacDonald *et al.*, 2000).

Statistical analysis

Data on the percent of infected leaves were Log-transformed. All data were analyzed for variance by Analysis of Variance (ANOVA). Significant differences among treatments were computed after Tukey HSD test at $P < 0.001$.

Results and discussion

Information on the susceptibility or resistance to OLS disease on olive cultivars in Palestine is based on field observations and farmer experience rather than on systematic studies. Assessment of disease is usually expressed by incidence and severity. "Disease incidence is the proportion (0 to 1) or percentage (0 to 100) of diseased entities within a sampling unit. For many plant diseases, only disease severity estimates are considered to give an accurate indication of their effects on the plants or of the efficacy of control treatments. Estimates of severity are frequently based on lesion area but may also be based on lesion number (Seem, 1984).

This study showed variation of OLS disease occurrence on individual olive cultivars grown closely to each other. Except for Nabali Mohassan, it was found that development of OLS disease over the time of the experiment did not differ between cultivars F=0.17, df=6, 83, p>0.983 and F=1.56, df=6, 83, p>0.169 for disease Incidence and severity, respectively (Figures 1 and 2). In Nabali Mohassan cultivar, the disease incidence and severity recorded in April (63.2 and 33.6) were significantly higher than that in October (24.3 and 19.8 F=2.4, df=6, 20, p>0.083 and F=4.6, df=6, 20, p>0.009 for both disease incidence and severity, respectively (Figures 1 and 2). Cultivars vary in their susceptibility as shown by the assessment and screening of OLS incidence and severity (Table 1 and Figure 3). Cultivars Nabali Mohassan, Nasohi Jaba`a 1, Nasohi Jaba`a 2 and Nabali Baladi showed the highest incidence and severity (49.7% and 28.9%, 11.9% and 18.3%, 9.0% and 14.8% and 8.6% and 20.4% (F=59.4, df=11, 251, p>0.0001and F=13.9, df=11, 251, p>0.0001) for both OLS incidence and severity, respectively. As shown in table 1, Barouni was the highest resistant cultivar (1 and 3.6% disease incidence and severity; respectively). Other cultivars (that Talmasani, Chemlali, Arbequino, Frantoio, Coca) were moderately resistant to OLS disease (Table 1).

The variation in susceptibility of olive cultivars under field is due to the effect of the environmental conditions such as temperature, RH and light (Obanor *et al.*, 2008a; Al-Khatib *et al.*, 2010). Previous findings also stated that the incidence and severity of OLS disease depend greatly on cultivar susceptibility and weather conditions

(Salman *et al.*, 2011; Sanei and Razavi, 2011; MacDonald *et al.*, 2000). Infections may occur throughout the year, except during hot and dry summers (Razavi and Jahany, 2009).

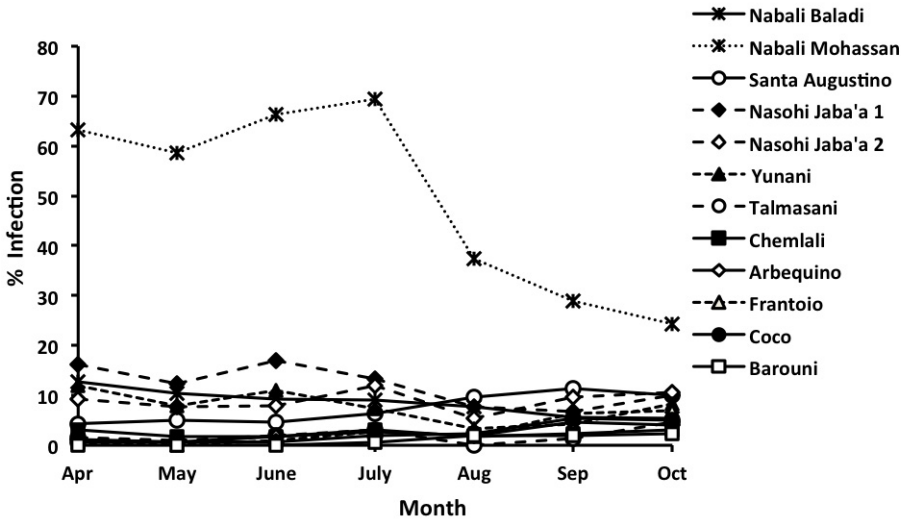

Figure 1 - Disease incidence (% of Infection) of Olive Leaf Spot (OLS) in different cultivars in Qabatyia station / Jenin district area. Data was pooled across all months for each cultivar for the period from April until October.

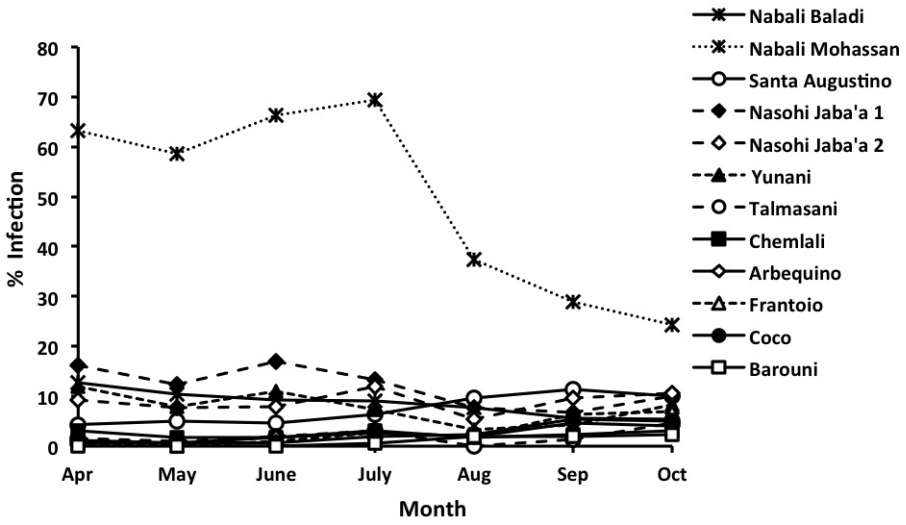

Figure 2 - Disease Severity (%) of Olive Leaf Spot (OLS) in different cultivars in Qabatyia station / Jenin district area. Data was pooled across all months for each cultivar for the period from April until October.

Table 1 - Effect of cultivars on Olive Leaf Spot Infection and Severity at Qabatyia Station in Jenin district area. These Data for 7 Months (April, May, June, July, August, September and October). Data in the same columns followed by the same letter are not significantly different at P ≤ 0.001 after Tukey's HSD test.

Cultivars	*%Incidence ± SD*	*%Severity ± SD*
Nabali Baladi	8.6 ± 2.4 ^{bc}	20.4 ± 2.3 ^{ab}
Nabali Mohassan	49.7 ± 6.0 ^{a}	28.9 ± 2.6 ^{a}
Santa Augustino	7.3 ± 2.7 ^{bc}	12.8 ± 1.9 ^{bcd}
Nasohi Jaba'a 1	11.9 ± 3.7 ^{b}	18.3 ± 2.0 ^{bc}
Nasohi Jaba'a 2	9.0 ± 2.0 ^{bc}	14.8 ± 2.0 ^{bc}
Yunani	7.8 ± 2.9 ^{bc}	14.2 ± 3.0 ^{bc}
Talmasani	1.9 ± 1.4 ^{c}	9.1 ± 3.4 ^{cd}
Chemlali	2.4 ± 0.6 ^{c}	10.9 ± 2.0 ^{cd}
Arbequino	2.4 ± 1.4 ^{c}	10.5 ± 3.4 ^{cd}
Frantoio	2.8 ± 2.6 ^{bc}	11.8 ± 5.0 ^{bcd}
Coca	2.4 ± 1.9 ^{c}	9.1 ± 3.2 ^{cd}
Barouni	1.0 ± 1.0 ^{c}	3.6 ± 3.5 ^{d}

The optimal weather conditions for the onset of the disease (relative humidity over 80%) occurred from January to June. Higher temperatures (> 20°C) were recorded during May to August. The decrease in temperature in October enables the outbreak of the OLS disease incidence again in all tested cultivars except the susceptible "Nabali Mohassan" cultivar (Figure 2). The highest level of disease severity in most tested cultivars was recorded in June (Figure 4b).

The infection of olive leaves with S. oleagina as well as other foliar fungal pathogens is normally influenced by temperature and wetness duration (Viruega et al., 2011; Viruega and Trapero, 1999). When wetness was not a limiting factor, infection occurs between 5 and 25°C. The effect of temperature in our study was minor because the temperature was the same during all assessment dates. The infection reached the highest values in all cultivars during June (25°C), July (26.6°C), and August (27.6°C) and then decreased in October (23.3°C). As determined by Obanor *et al.* (2008a; 2011), the optimum temperature for infection was 15°C, this might explain the

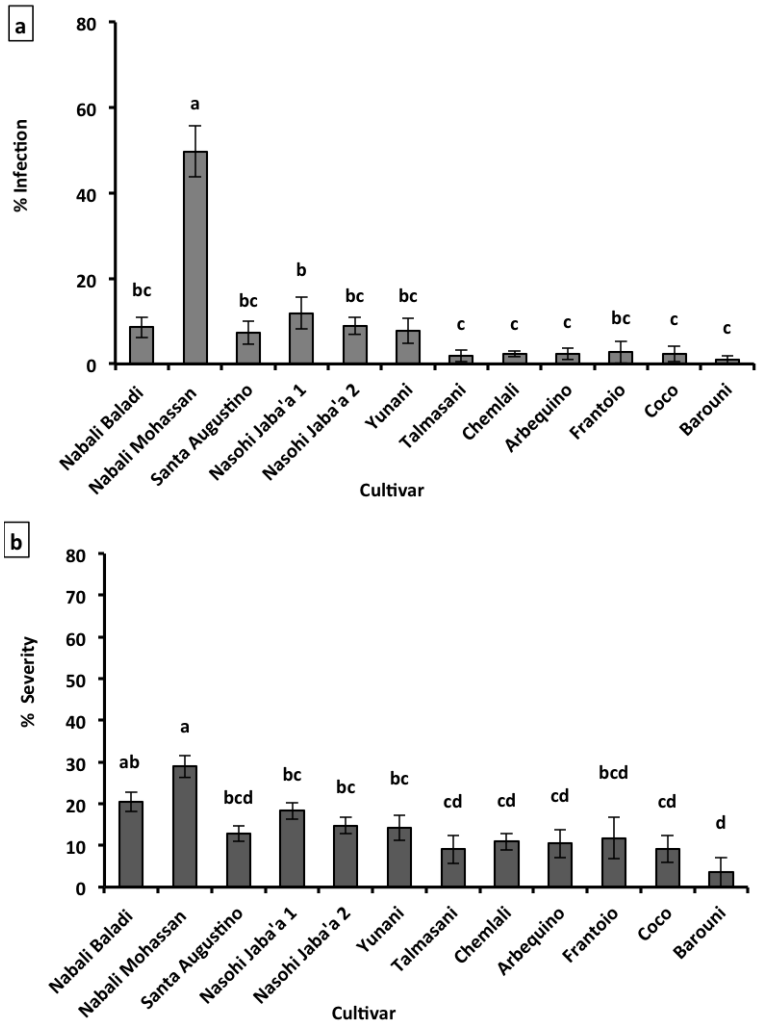

*Figure 3 - Disease incidence (% of Infection) (**a**) and Severity (**b**) of Olive Leaf Spot (OLS) in different cultivars in Qabatyia station / Jenin district area. Data was pooled across all months for each cultivar for the period from April until October. Data with different letters are significantly different after Tukeys HSD test using ANOVA at P < 0.001.*

increased rate of infection and severity in all cultivars during October (Figures 1, 4). Guechi and Girre (1994) reported that the most prevalent period for the OLS infection occurred from late autumn to spring. Minor significance of the disease was obvious in the period from the beginning of July until the middle of November. Obanor *et al.* (2005) and Graniti (1993) also reported that the infection with *S. oleagina* occurs

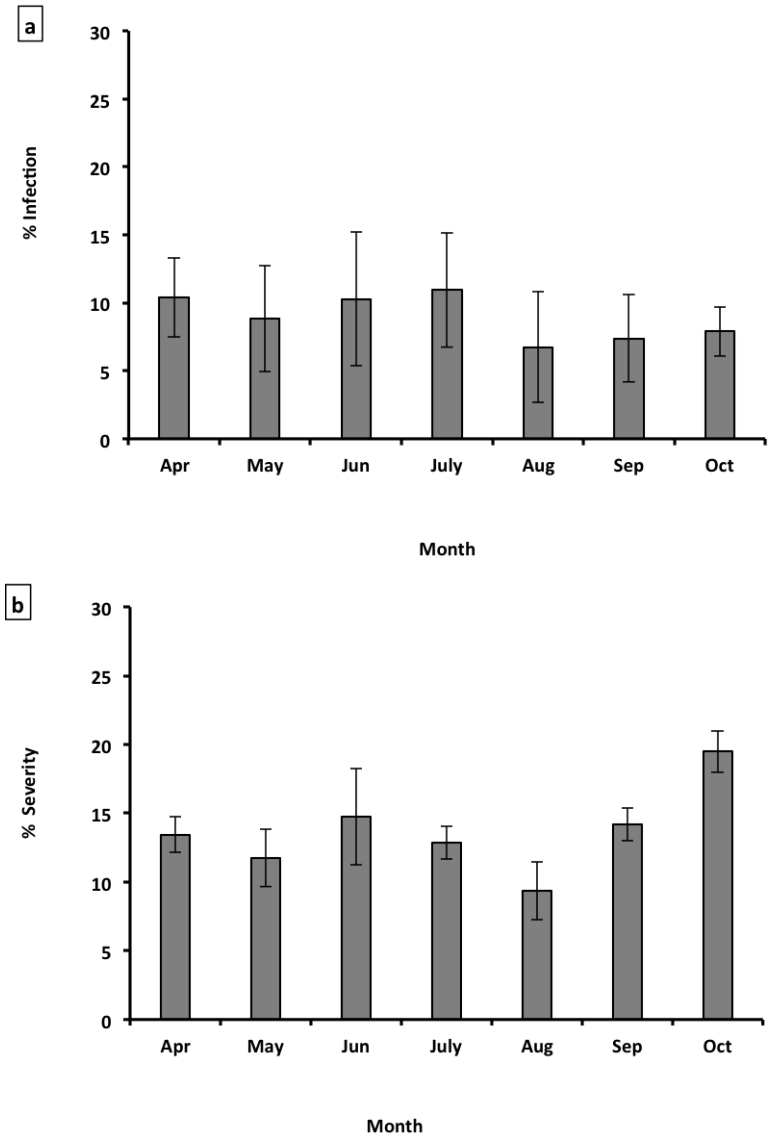

*Figure 4 - Disease incidence (% of Infection) (**a**) and Severity (**b**) of Olive Leaf Spot (OLS). Data was pooled across all cultivars for each month from April until October, no differences were detected after Tukeys HSD test using ANOVA at $P < 0.001$.*

during autumn to early spring and the pathogen is dormant during hot, dry summers. *S. oleagina* survives during summer as mycelium in the lesions on living leaves.

Tested olive trees of similar age/size and canopy density were selected in order to

reduce the variability of OLS disease between trees. Moreover, the time of assessment had no significant effect on OLS disease levels and there no significant differences (F=0.17, df=6, 83, p 0.983 and F=1.56, df=6, 83, p 0.169) between months was recorded for OLS incidence and severity, respectively (Figure 4). Extensive studies have been conducted to determine the effects of several variables on infection, lesion development, yield losses, and management of diseases caused by S. oleagina (Rhouma *et al.*, 2012; Viruega *et al.*, 2011; Obanor *et al.*, 2011; Obanor *et al.,* 2008a; Guechi and Girre, 1994). However, the influence of environmental and host conditions are not well known.

These factors were very highly correlated $R^2 = 0.84$. The number of lesions found on the leaves depended on disease severity ($y = 0.42x + 9.3$, $P \leq 0.0001$, $R^2 = 0.84$, y = disease severity and x = disease incidence) (Figure 5). Incidence data (% infected leaves) can be easily recorded with greater accuracy than severity data (number of lesions/leaf), particularly when leaves are wet. Therefore the % infected leaves should be used for assessing the efficacy of any control measures tested in the future.

There is a high correlation ($P<0.001$) between number of infected leaves per tree, numbers of lesions per leaf and diseased leaf areas, and OLS lesions are known to expand very slowly (Shabi *et al.*, 1994). For this reason assessment of disease severity as the number of lesions per leaf was considered to be valid for this study (Agrios, 2005). The relationship between disease incidence (% leaves infected) and severity was determined in many reports (Obanor *et al.*, 2005; Salman *et al.*, 2011).

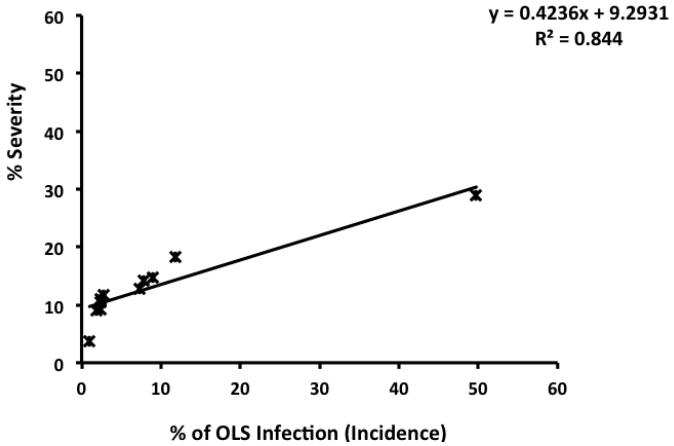

Figure 5 - Relationship between Olive Leaf Spot (OLS) incidence (% infected leaves) and Severity of OLS (number of lesions/leaf) across all cultivars and assessment times determined from April tell October in Qabatyia station / Jenin district area. There is a correlation between incidence and severity ($y = 0.42x + 9.3$, $P \leq 0.0001$, $R^2 = 0.84$, y = disease severity and x = disease incidence).

The present study showed variable infection rates with OLS among different olive cultivars with percentage infected leaves ranging between 1 and 50%. Resistant cultivars usually show no or very few leaves with visible spots. According to Benitez *et al.* (2005), evaluation of susceptibility cannot be based on the latent infections and must be therefore determined using visible infections. Therefore, a high variability in susceptibility of olive cultivars to the disease was determined.

Conclusion and Recommendation

This study was the first that evaluate the infection of OLS on different cultivars of olive trees in Palestine. Further monitoring is needed to better understand the disease incidence to set up a suitable control strategy. The relative resistances/ susceptibilities of olive cultivars to OLS require thorough evaluation in Jenin area. The weather conditions in Jenin district are most suitable for OLS development with an annual rainfall of 566 mm, average temperature 27 ^{o}C and relative humidity range 60-85%.

The studied olive trees were grown closely to each other in Qabatya station creating more suitable condition for disease development. For this reason, pruning (increasing aeration and reducing humidity) is highly recommended for better control of the disease. In addition to that chemical treatment should be applied directly after harvest season followed by an additional spray in March and June (the peak of disease development).

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